

5 **Claims**

1. A primer designed for use with mRNA comprising a 5' sequence based on a 5' consensus region of the mRNA and a 3' sequence capable of hybridising to a 3' region of the mRNA.
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2. A primer according to claim 1, wherein the primer 5' sequence comprises sequence identical or similar to sequence of the mRNA 5' consensus region.
- 15 3. A primer according to claim 1 or 2, wherein the primer 3' sequence comprises sequence complementary to the mRNA 3' region.
4. A method for generating a cDNA molecule which comprises reverse transcription of mRNA using a primer according to any of claims 1 to 3.
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5. A method for recovery of cDNA which comprises generating cDNA using a method of claim 4, and PCR amplification of the cDNA using a single primer.
- 25 6. A method according to claim 5, wherein the single primer comprises a 5' sequence based on the mRNA 5' consensus region.
7. A kit for a method according to claim 5, which comprises a supply of primer according to any of claims 1 to 3, and one or more of a supply of dNTP, a supply of reverse transcriptase, a supply of ribonuclease inhibitor, buffer, RNase-free water.
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- 35 8. A kit for a method according to claim 6, which comprises a kit according to claim 7 supplemented with one or more PCR components such as DNA polymerase, PCR buffer, PCR primer(s) and dNTPs .
9. A method for recovery of cDNA from mRNA, said method comprising

5 (a) reverse transcription (RT) of mRNA using a primer which includes a sequence identical or similar to the 5' consensus region of the mRNA and which includes a sequence capable of hybridising specifically to the 3' region of the mRNA, followed by,

10 (b) polymerase chain reaction (PCR) using a single primer to amplify the cDNA.

10. A method according to claim 9 wherein in (b) the cDNA is present as a mixture of molecules or as a single molecule.

15 11. A method according to claim 10, wherein the mRNA is at least partially denatured before the RT reaction, preferably by heat treatment or a chemical method.

20 12. A method for recovery of DNA fragments from mRNA, said method comprising:

(a) heating a sample comprising mRNA, followed by,

(b) RT using a primer which includes a sequence identical to or similar to the sequence at the 5' consensus region of the mRNA, followed by,

(c) PCR using a single primer to amplify the ss cDNA obtained in (b).

25 13. A method according to claim 12, wherein in (c) the ss cDNA is present as a mixture of molecules or as a single molecule.

30 14. A method according to any one of claims 9 to 13, wherein the RT primer used is an oligonucleotide or mixture of oligonucleotides in which a 3' sequence is complementary to a 3' region of the template mRNA and in which the 5' sequence comprises sequence identical or similar to the 5' consensus region of the mRNA.

35 15. A method according to any one of claims 9 to 14, wherein the RT primer used is an oligonucleotide or mixture of oligonucleotides in which a 3' sequence is complementary to a 3' region of the template mRNA which may

5 optionally include part of the poly A tail, and in which the 5' primer region has a sequence similar or identical to the 5' region of the mRNA.

10 16. A method according to any one of claims 9 to 15 wherein, the RT primer used is an oligonucleotide or mixture of oligonucleotides in which a 3' primer region is complementary to a 3' region of the template mRNA and in which the 5' primer region has a sequence similar or identical to a 5' region of the mRNA including the sequence for one or more of the transcriptional start site, regulatory elements, kozak sequence, translational start codon, any part of the translated sequence or any family specific consensus sequence found in 15 the 5' region.

20 17. A method according to any one of claims 9 to 16, wherein the RT primer used is an oligonucleotide or mixture of oligonucleotides in which a 3' primer region is complementary to a 3' region of the template mRNA which 3' region may optionally include a part of the poly A tail (i.e. the 3' region may include, but not consist solely of, a part of the poly A tail), and in which the 5' primer sequence comprises a sequence similar or identical to the 5' consensus region of the mRNA including the sequence for one or more of the transcriptional start site, regulatory elements, kozak sequence, translational 25 start codon, any part of the translated sequence or any family specific consensus sequence found in the 5' consensus region.

30 18. A method according to any one of claims 9 to 17, wherein the single primer used for PCR is identical, overlapping with or similar to, the 5' sequence of the RT primer used.

19. A method for RT-PCR recovery of cDNA from mRNA in ribosome display complexes, said method comprising:

35 (a) RT using a primer comprising a 5' sequence which is similar or identical to the 5' consensus region of mRNA and a 3' primer region sequence complementary to a 3' region of mRNA, followed by,

 (b) PCR using a single primer to amplify the ss cDNA obtained in (b).

5 20. A method according to claim 19 wherein in (b), the ss DNA is present as mixture or a single molecule.

10 21. A method according to claim 19 or claim 20, wherein the ribosome display complexes are treated before RT to make mRNA accessible to primer(s), preferably by heating and/or by a chemical method.

15 22. A method for recovery of DNA fragments from mRNA in ribosome display complexes, said method comprising:
(a) heating of ribosome complexes, followed by,
(b) RT using a primer which includes a sequence identical to or similar to the sequence at the 5' consensus region of the mRNA, followed by,
(c) PCR using a single primer to amplify the ss cDNA obtained in (b).

20 23. A method according to claim 22 wherein in (c) the ss cDNA is present as mixture or a single molecule.

25 24. A method according to any one of claims 19 to 23, wherein the ribosome display complex is an antibody-ribosome-mRNA complex.

30 25. A method according to any one of claims 19 to 24, wherein the 5' sequence of the RT primer is a sequence similar to or identical to the 5' consensus region of the mRNA, including the sequence of one or more of the transcriptional start site, regulatory elements, kozak sequence, translational start codon, any part of the translated sequence or any family specific consensus sequence found in the 5' region.

35 26. A method according to any one of claims 19 to 25, wherein the single primer used for PCR is identical, overlapping with or similar to, the 5' sequence of the primer used for the RT reaction step.

27. A method or kit according to any one of claims 4 to 26, wherein the primer used for RT is HuRT (SEQ ID NO: 3).

5 28. A method or kit according to any one of claims 4 to 27, wherein the primer used for PCR is Kz1 (SEQ ID NO: 1).

10 29. A method according to any preceding claim wherein the whole or a part or parts of the method is automated.

15 30. A method according to any preceding claim wherein the display of the mRNA protein complex is automated.

31. A method according to any preceding claim wherein the RT reaction(s) is automated.

15 32. A method according to any preceding claim wherein the PCR reaction(s) is automated.

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